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Genetic Control of T-Cell Proliferative Responses to Poly(Glu^{40}Ala^{60}) and Poly(Glu^{51}Lys^{34}Tyr^{15}): Subregion-Specific Inhibition of the Responses with Monoclonal Ia Antibodies

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Abstract. The relationship between *Ir* genes and Ia antigens was studied in the T-cell proliferative responses to two synthetic polypeptides poly(glu^{40}ala^{60}) (GA) and poly(glu^{51}lys^{34}tyr^{15}) (GLT^{15}). The response to GA was found to be controlled by an *Ir* gene in the *I-A* subregion, whereas the anti-GLT^{15} response was shown to be under dual control, one *Ir* gene mapping probably in the *I-A* subregion, and the other in the *I-E* subregion. We obtained two different lines of evidence suggesting identity of *Ir* and *Ia* genes. First, the presence of certain serologically identified allelic forms of the I-A-encoded A molecule correlated with the responder status to GA both in inbred strains and in B10.W lines, the latter carrying wild-derived *H-2* haplotypes. Thus the Ir and Ia phenotypes were not separable in strains of independent origin. Second, the anti-GA response was completely inhibited by monoclonal antibodies against determinants on the A molecule (Ia.8, 15, and 19), but not by a monoclonal antibody against a determinant on the E molecule (Ia.7). In contrast, the anti-GLT^{15} response was only inhibited by a monoclonal antibody against the E molecule, but not by antibodies against the A molecule. Our data support the hypothesis that Ia antigens, as restriction elements for T-cell recognition, may in fact be the phenotypic manifestation of *Ir* genes.

troduction

ıe so-called *I* region of the mouse *H-2* complex governs a series of traits, of which e most intensively studied are the control of the immune response by *Ir* genes and e control of cell-surface molecules by *Ia* genes (reviewed by Benacerraf and

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McDevitt 1972, Shreffler and David 1975). For several years after their discover the *Ir* remained genes with a function but without a product, whereas the remained genes with a product but without a function. It was not until 1974, the ye in which it was discovered that cytolytic T cells recognize antigens in the context class I major histocompatibility complex (MHC) molecules (Zinkernagel ar Doherty 1974), that a functional link between the *Ir* and *Ia* genes was suggeste One could postulate, as we (Klein 1976) and others (Doherty and Zinkernagel 197 did, that the *Ir* genes function on a similar principle to the class I genes, and that tl Ir phenomenon reflects the recognition by helper T cells of antigens in the context class II MHC molecules. This interpretation made much more sense than tl original hypothesis, according to which the *Ir* genes coded for the T-cell recept (Benacerraf and McDevitt 1972).

Since then data have accumulated supporting the new interpretation. First, has been demonstrated that helper T cells do recognize antigen in the context I-region products (Erb and Feldmann 1975, Sprent 1978). Second, Ir-gene produc have recently been shown to be expressed on the antigen-presenting cell but not o the responding T cell, and they appear to function as restricting elements for T ce recognition (Schwartz et al. 1979 b, Longo and Schwartz 1980). In this report w present a third line of evidence by showing subregion-specific blocking of *Ir*-gen controlled T-cell responses by monoclonal Ia antibodies. Taken together, these dat provide strong evidence that Ia antigens and Ir-gene products are identical.

Materials and Methods

Mice. All inbred and congeneic mouse strains were obtained from our colony at the Max Planck Institut for Biology. Twelve to 16 week old female and male mice were used. The strains and their alleles at *H-* loci are given in Tables 1, 2 and 4.

Antigens and mitogen. Random copolymers of amino acids poly(glu^{40}ala^{60}) (GA) an poly(glu^{51}lys^{34}tyr^{15}) (GLT^{15}) were synthesized by polymerization of N-carboxy-α-amino acid anhy drides (Katchalski and Sela 1958); they were purified by extensive dialysis and lyophilized. Before use, th antigens were dissolved in distilled water adjusted to pH 8.1 with Tris buffer (20 mM), aliquoted, an stored at −20° C. For tissue culture, the antigens were diluted in RPMI-1640 (Gibco, BCK Biocult Chemie, Karlsruhe, FRG) to a concentration of 0.2–0.4 mg/ml, and sterilized by γ-irradiation (3000 R) Concanavalin A (Con A, Deutsche Wellcome, Burgwedel, FRG) was dissolved in RPMI at 0.1 mg ml sterilized by filtration through 0.45 μm pore size filters (Millipore), aliquoted, and stored at −20° C.

Monoclonal antibodies. Hybridoma antibodies in ascites form were a gift from Dr. G. Hämmerling (German Cancer Research Center, Heidelberg, Federal Republic of Germany). The following antibodies were used: B15-124R4 (anti-Ia.m2), 13/18 (anti-Ia.m7), B22-277R19 (anti-Ia.m8), 17/227.R7 (anti-Ia.m5 recognizing Ia.15), H116-32.R5 (anti-Ia.m6 recognizing Ia.19), B22-249R1 (anti-H-2.m2, or anti-H-2D^b), and H100-30/3 (anti-H-2.m5) (Lemke et al. 1979). To remove low molecular weight, nonspecific inhibitory substances, the ascites antibodies were fractionated by ultrafiltration, using XM-100A Amicon membrane filters (Klein et al. 1977). The antibodies were stored at −70° C and sterilized by γ-irradiation before use.

Immunizations. The antigens were diluted in Hanks' balanced salt solution (HBSS, Gibco) and emulsified with equal volumes of complete Freund's adjuvant (Difco, Hedinger, Stuttgart, FRG). One hundred micrograms of antigen in 50 μl volume were injected subcutaneously at the base of the tail (Alkan 1978).

ıle 1. Proliferative responses of standard *H-2* haplotypes to GA

ıin	*H-2* haplotype	Δcpm range	Stimulation index range	No. of significant responses per total no. of experiments	Responder status
BL 10 BL·6 .B.B 52	*b*	1.174–118.271	1.3–33.0	6/7	+
.B/c A 2 -H-2^{d}H-4^b	*d*	4.080–117.002	2.3–10.3	7/7	+
A M	*f*	4.266–50.871	1.8–12.5	8/8	+
WB	*j*	(−1.253)	0.4	0/1	−
BR	*k*	383–37.517	1.3– 9.3	20/22	+
.NB P	*p*	(−3.541)–(−72)	0.4– 0.9	0/4	−
Q	*q*	(−389)–1193	0.9– 1.9	1/4	−
RIII	*r*	(−10.384)–5.021	0.3– 2.8	0/5	−
5 /	*s*	(−618)–859	0.6– 1.4	0/3	−
PL	*u*	(−1.293)	0.5	0/1	−
SM	*v*	(−1.367)	0.5	0/1	−

h-node cell proliferative assay. The proliferative assay described by Alkan (1978) was employed with modification. Nine to 11 days after immunization, the draining lymph nodes (para-aortic and ıal) were removed aseptically, and single cell suspensions were prepared from the pooled lymph of two to three mice. After two washes, the cells were resuspended in culture medium consisting of I-1640 supplemented with 5% heat-inactivated horse serum (Gibco), penicillin 100 units/ml, omycin 100 μg/ml, and L-glutamine (2mM final concentration). The cells were cultured in 0.2 ml volumes on microculture plates (Falcon, LS Labor Service, München, FRG). Optimal responses obtained with 4×10^5 viable cells per well in the presence of 20 μg antigen. The controls consisted of ltures without antigen, and cells with 5 μg Con A per culture. Peak proliferative responses occurred y 3, and were measured by thymidine incorporation, after adding 2 μCi of ^{3}H-methyl thymidine England Nuclear) for the last 16 to 24 h of culture.

he proliferation was mediated by T cells, since pretreatment of the lymph-node cells with anti-2 plus rabbit complement resulted in a markedly diminished response (data not shown).

or inhibition of the response, monoclonal antibodies at the appropriate dilutions were included in ne volume (0.2 ml/culture) of medium, and were present throughout the culture period. After 3 days tures were harvested in a semiautomated harvestor, and thymidine uptake was determined using a ıann scintillation counter. All determinations were done in triplicate, and data are expressed as standard deviation (S. D.). Significant responses over the background (i. e., cells without antigen) alculated using the Student's *t* test.

Table 2. Proliferative responses of B10.W lines to GA

Line	Alleles at *H-2* loci				Δcpm range	Stimulation index range	No. of significant responses per no. of experiments	Responder status
	K	*A*	*E*	*D*				
BUA16	*w16*	*w16*	*7*	*k*	−1232	0.5	0/1	−
BUA19	*w16*	*w16*	*7*	*k*	−370–(−275)	0.8–0.9	0/2	−
CAA2	*f*	.	*7*	*w11*	−125–1881	0.9–1.3	0/2	−
CAS1	*w23*	.	.	*w23*	−94–(−11)	0.5–1.0	0/2	−
CAS2	*w17*	*w17*	*0*	*w3*	4573–23.048	1.9–2.3	3/3	+
CHA2	*q*	*k*	*7*	*w26*	5271–6340	5.3–7.4	3/3	+
CHR51	*d*	.	*7*	*w18*	−483–82	0.6–1.0	0/3	−
DRB62	*v*	*v*	*7*	*w10*	−383–9	0.7–1.0	0/2	−
GAA20	*w4*	*w4*	*7*	*w4*	3631	2.8	1/1	+
KPA44	*d*	*w16*	*7*	*w24*	−76–648	0.9–1.4	0/2	−
KPA132	*v*	*v*	*7*	*w1*	−220–105	0.7–1.0	0/2	−
KPB128	*s*	*s*	*7*	*b*	−330–697	0.6–1.5	0/3	−
LIB18	*w16*	*w16*	*7*	*w8*	−24–1570	0.9–1.3	0/2	−
LIB55	*w13*	*w13*	*7*	*w13*	16.886–21.570	4.3–7.5	8/8	+
SAA48	*w3*	*w3*	*7*	*w3*	−140–(−52)	0.9–1.0	0/2	−
SNA57	*v*	*v*	*7*	*w10*	−203–737	0.9–1.0	0/2	−
SNA70	*k*	*k*	*7*	*w8*	2.690–7351	2.9–4.6	3/3	+
STA10	*w13*	*w13*	*7*	*w13*	57–187	1.1–1.5	0/7	−
STA39	*w3*	*w13*	*7*	*w13*	26.026	14.4	1/1	+
STA62	*w27*	*b*	*7*	*w27*	4.174–13.170	6.4–9.6	2/2	+
STC77	*d*	.	*7*	*w14*	−498–(−205)	0.4–0.8	0/2	−
STC90	*w15*	*w15*	*7*	*w15*	2112–7238	3.0–4.3	3/3	+
WOA105	*v*	*v*	*7*	*w10*	−96–759	0.6–1.5	0/2	−

Results

Genetic control of the T-cell proliferative response to GA. It has been previously demonstrated that the antibody response of inbred mice to GA is controlled by an *H-2*-linked immune response (*Ir*) gene (Merryman and Maurer 1976). Since T-cell responsiveness to this antigen has not been investigated, we first determined the responses of standard *H-2* haplotypes in the lymph-node T-cell proliferation assay (Table 1). All strains classified previously as nonresponders (*H-2* haplotypes *j*, *p*, and *q*) by the antibody assay proved also to be nonresponders at the T-cell level. Out of the six strains that produced antibody to GA, four (*H-2* haplotypes *b*, *d*, *f*, and *k*) were responders, and two (*H-2* haplotypes *r* and *s*) were nonresponders in the proliferation assay. Further, two strains (*H-2* haplotypes *u* and *v*) not tested previously for antibody response proved to be nonresponders in terms of T-cell proliferation. Thus the patterns of responsiveness as determined by the two different assays were almost identical with two exceptions, the reason for which is presently. It was also noted that the magnitude of the proliferative response was different in strains carrying the same *H-2* haplotype on various backgrounds. A similar phenomenon was observed in T-cell responses to several antigens (Suzuki et al. 1979). However, the influence of non-*H-2* genes has never been sufficient to change

the responder status of the strain (i.e., to turn a responder *H-2* haplotype in to a nonresponder, or vice versa).

To locate the gene controlling the anti-GA response within the *H-2* complex, we made use of the B10.W congenic lines, which carry different wild-derived *H-2* haplotypes on the B10 background. Many of these lines have alleles at different *H-2* loci, that are serologically indistinguishable from those of inbred strains (Wakeland and Klein 1979). These particular lines can thus be considered natural recombinants of inbred *H-2* alleles. Testing of the proliferative response to GA on a panel of B10.W mice is shown in Table 2. The data clearly indicate that all strains that carry a responder inbred allele (*k* or *b*) at the *A* locus are responders, and those with a nonresponder inbred A allele (*s* or *v*) are nonresponders. Particularly informative in this respect are the CHA2 and STA62 strains, which carry responder alleles only at the *A* locus. Furthermore, some correlation can be demonstrated between the presence of certain wild alleles at the *A* locus, and responsiveness to GA. For example, all four strains which have *w16* at the *A* locus are nonresponders, and two out of three strains with *w13* at the *A* locus are responders. In the latter instance, so far undetected differences in the *w13* allele in responder and nonresponder strains may account for the discrepancy observed. Further responder alleles identified in B10.W lines were *w4*, *w15*, and *w17*. These results are consistent with earlier mapping data on inbred recombinant strains, which localized the gene controlling the anti-GA antibody response to the *I-A* (or *I-B*) region (C. F. Merryman and P. H. Maurer, unpublished observations). In addition, our results extend the pattern of responsiveness based on typing the inbred strain panel, by including responder and nonresponder B10.W strains with noninbred (wild) alleles at the *A* locus.

Genetic control of the T-cell proliferative response to GLT^{15}. The T-cell proliferative responses of standard *H-2* haplotypes to GLT^{15} are summarized in Table 3. In agreement with the strain distribution of antibody response to this antigen (Merryman and Maurer 1975), the *d* and *r* haplotypes were responders. However, in contrast to the positive antibody response, *H-2*^{q} gave a significant proliferative response only in one out ot three experiments. Schwarz and co-workers (1979a) made similar observations using the closely related antigen GLT^{5}, of which *H-2*^{q} strains required higher immunizing doses for a significant T-cell proliferation than other responder haplotypes.

As to the location of the gene(s) controlling the response to GLT^{15} no data have so far been available. It was, however, reasonable to assume that two complementing genes operate in this response, as has been shown for responses to the structurally similar GLT^{5} and GLØ (Dorf and Benacerraf 1975, Schwartz et al. 1976 b and 1979 a). Data consistent with two-gene control of the T-cell response to GLT^{15} are shown in Table 4. The presence of responder alleles at *H-2* loci either right or left of *I-J* was insufficient to produce a response. However, two nonresponder strains could complement each other in both the *cis* and the *trans* position to become a responder. Although the genes could not be located by formal mapping for lack of the appropriate recombinants, data obtained in the *GLØ* system (Dorf and Benacerraf 1975, Schwartz et al. 1976 b) together with our antibody-inhibition experiments to be presented in the next section indicate that the relevant genes map to the *A* and *E* regions.

Table 3. Proliferative responses of standard *H-2* haplotypes to GLT[15]

Strain	*H-2* haplotype	Δcpm range	Stimulation index range	No. of significant responses per total no. of experiments	Responder status
C57BL/6 BALB.B B6.K2	*b*	(−432)–10.980	0.4–2.5	2/15	−
BALB/c B10-H-2^{d}H-4^{b}	*d*	8.621–208.653	5.8–20.0	4/4	+
A.CA B10.M	*f*	(−1.778)–3546	0.7–2.4	0/5	−
B10.WB	*j*	(−1.452)	0.3	0/1	−
B10.BR CBA	*k*	(−1.359)–(−259)	0.5–0.7	0/3	−
B10.P	*p*	(−1.599)	0.3	0/1	−
B10.Q	*q*	1.007–36.136	1.7–7.2	1/3	+ − ?
B10.RIII	*r*	58.555–157.632	24.7–42.0	2/2	+
B10.S A.SW	*s*	(−2.501)–581	0.4–1.9	0/3	−
B10.PL	*u*	(−320)	0.7	0/1	−
B10.SM	*v*	(−594)	0.7	0/1	−

Table 4. Gene complementation in the T lymphocyte proliferative response to GLT[15]

Strain	Alleles at *H-2* loci						cpm	Stimulation index
	K	*A*	*J*	*E*	*S*	*D*		
Balb/c	*d*	*d*	*d*	*7*	*d*	*d*	8621	5.8
B10.GD	*d*	*d*	*d*	*0*	*b*	*b*	−757	0.5
B10.M(11R)	*f*	*f*	*f*	(7)	*f*	*d*	−380	0.7
B10.A	*k*	*k*	*k*	*7*	*d*	*d*	− 80	0.9
B10	*b*	*b*	*b*	*0*	*b*	*b*	− 82	0.9
B10.A(5R)	*b*	*b*	*k*	*7*	*d*	*d*	8793	5.6
AKR	*k*	*k*	*k*	*7*	*k*	*k*	−310	0.8
(AKR × B10)F_1	*k* *b*	*k* *b*	*k* *b*	*7* *0*	*k* *b*	*k* *b*	2677	3.2

Inhibition of the T-cell proliferative response to GA and GLT[15] with monoclonal Ia antibodies. The induction of antigen-specific T-cell proliferation involves interaction of T cells with macrophage-like antigen-presenting cells (Waldron et al. 1973). The T-cell-macrophage interaction is controlled by the *I* region of MHC, and is governed by Ia antigenic determinants expressed on the macrophage but not on the T cell (Rosenthal and Shevach 1973, Erb and Feldmann 1975, Kappler and Marrack 1976, Yano et al. 1977, Thomas et al. 1977). These findings, together with

the known location of *Ir* genes within the *I* region (Shreffler and David 1975) strongly suggest that at least some of the *Ir* genes controlling antigen-specific T-cell proliferation may be manifested phenotypically as Ia antigens (Benacerraf 1978). Earlier studies using Ia alloantisera for the inhibition of *Ir*-gene-controlled T-cell proliferation have also pointed to this possibility (Schwartz et al. 1976a, 1978).

To show conclusively a correlation between the expression of Ia antigens and *Ir* genes, we used monoclonal antibodies recognizing Ia determinants on either the A or the E molecule (Lemke et al. 1979) for inhibition of the T-cell proliferative responses to GA and GLT^{15}, which are controlled by *Ir* genes in the *A* region and in the *A* plus *E* region, respectively. Antibody inhibition of the anti-GA response by five different responder strains is shown in Figure 1. A monoclonal antibody (B15-124R4) against Ia.m2 not expressed by any of the responder haplotypes did not inhibit-T-cell proliferation within a wide range of concentrations. The proliferation was not inhibited by 13/18, which recognizes Ia.m7 on the E_α chain, although this determinant was expressed by three out of the five responder strains tested. Similarly, B22-249R1, which recognizes H-2.m2 (the private determinant of $H\text{-}2D^b$), failed to inhibit the anti-GA response of T cells from B6 mice. However, B22-277R19 (anti-Ia.m8) and 17/277.R7 (anti-Ia.m5), each recognizing a determinant on the A molecule, caused strong inhibition of T-cell proliferation. Very small concentrations (0.01 to 0.1 percent) of antibodies were sufficient to cause significant blocking, and the degree of inhibition was dependent on the concentration of antibodies. The inhibitory capacity of the antibodies seemed to run parallel to their cytotoxic titer on the same strains (unpublished observations). Thus the *A*-region controlled response of T cells to GA is only inhibited by monoclonal antibodies against the Ia molecule encoded by the *A* region. The fact that the response of LIB55, which carries wild-type *H-2* alleles, was also inhibited by anti-Ia.m5, strengthens our conclusion that the relevant *Ir* gene is inseparable from Ia antigens.

Inhibition of the anti-GLT^{15} response of several strains with monoclonal antibodies is shown in Figure 2. In contrast to the anti-GA response, T-cell proliferation to GLT^{15} was only inhibited by 13/18, which recognizes Ia.m7 present on the E_α chain. Three different antibodies against the A molecule (anti-Ia.m5, m6, and m.8) failed to block the response, despite the expression of this molecule by the strains tested. The A molecule seemed to be irrelevant to the proliferative response also in the two responder B10.W strains (STA10 and LIB55), which carry wild-derived alleles at the *A* locus (see Table 2). Control antibodies such as anti-Ia.m2 (not expressed by the responding strains), and anti-H-2.m5 (expressed by B10.RIII and STA 10) did not interfere with the response. Thus the anti-GLT^{15} response, which is controlled by two complementing *Ir* genes in the *A* and *E* region was only inhibited with an antibody against the E molecule, and with none of the antibodies against the A molecule. This finding implies that the second *Ir* gene relevant to the response is most likely expressed as the E_β chain, which is also encoded by the *A* region, but is serologically distinct from the $A_\alpha A_\beta$ molecule (Jones et al. 1978, Lafuse et al. 1980).

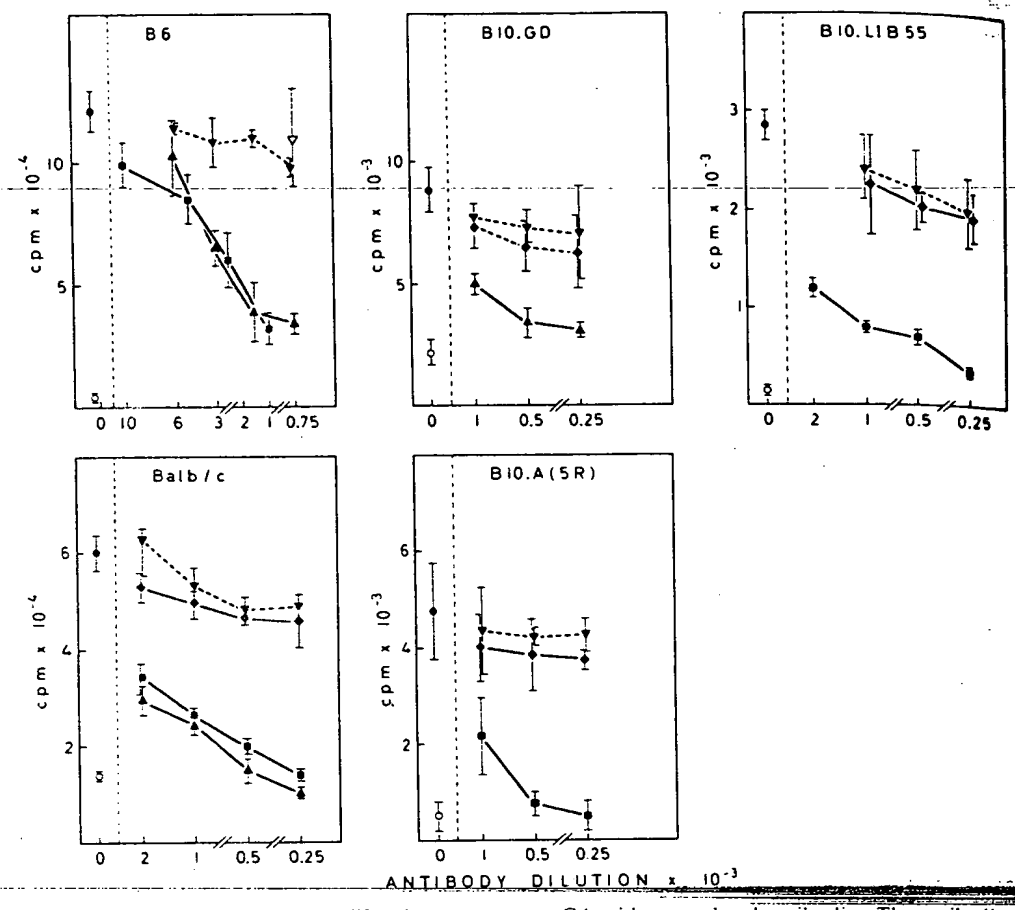

Fig. 1. Inhibition of T-cell proliferative responses to GA with monoclonal antibodies. The antibodies added to cultures were anti-Ia.m2 (▼), anti-Ia.m7 (◆), anti-Ia.m8 (△), anti-Ia.m5 (■), and anti-H-2.m2 (▽). The *solid line* indicates that the relevenat antigen is expressed, and the *dashed line* that it is not expressed by the responding strain. In the controls, proliferation was tested in the presence (●) or absence (○) of antigen, with no antibodies added to cultures. *Vertical bars* represent ±S. D.

Discussion

The data presented in this communication support the theory that *Ir* and *Ia* genes are identical, in two ways. First, we found that B10.W lines carrying *A* alleles indistinguishable serologically from those present in inbred strains, or other B10.W lines with certain wild-derived *A* alleles type as if carrying the same *Ir* allele when immunized with the synthetic polymer GA (Table 2). Thus, all strains – whether inbred or B10.W – carrying *k*, *b*, or *w13* at the *A* locus are responders to GA, whereas all strains carrying the *s*, *r*, or *w16* allele at the *A* locus are nonresponders. The one exception to this finding is strain B10.STA10 which has been serologically

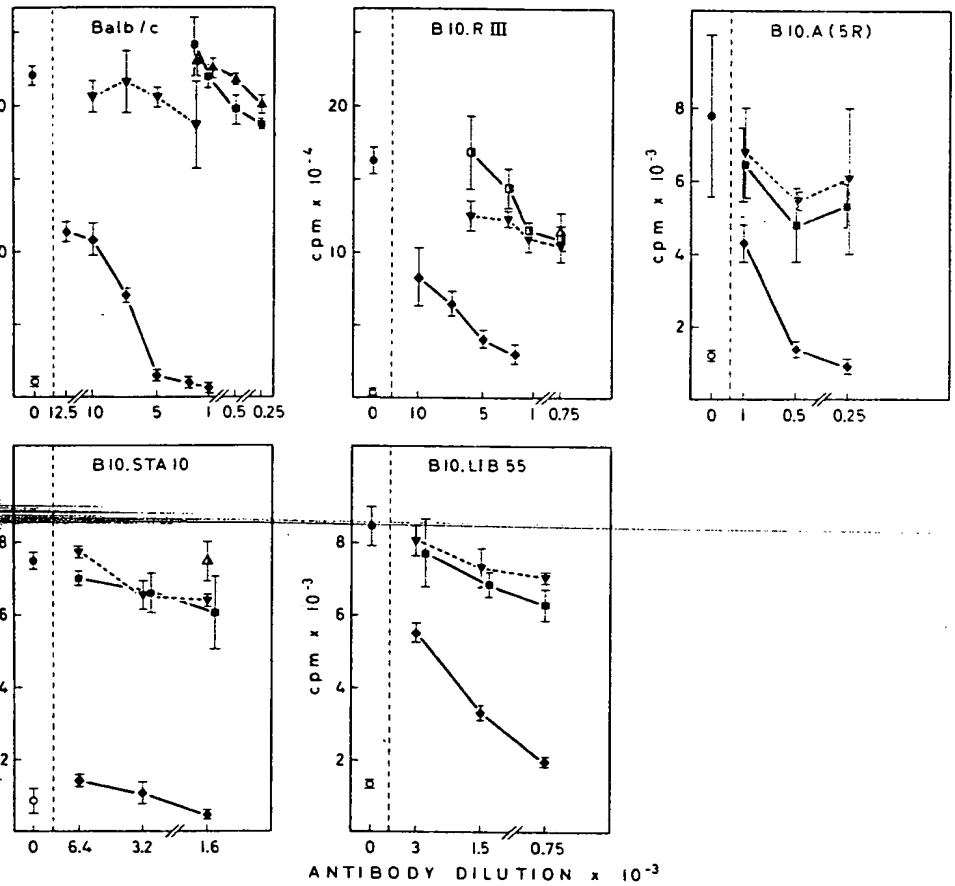

Fig. 2. Inhibition of T-cell proliferative responses to GLT[15] with monoclonal antibodies. The antibodies added to cultures were anti-Ia.m2 ( ), anti-Ia.m7( ), anti-Ia.m8 (▲), anti-Ia.m5 ( ), anti-Ia.m6 (□), and anti-H-2.m5 (△). The *solid line* indicates that the relevant antigen is expressed, and the *dashed line* that it is not expressed by the responding strain. In control cultures, proliferation was measured without antibody, in the presence (○) or absence (●) of antigen. *Vertical bars* represent ± S.D.

typed as A^{w13} because it possesses antigen Ia.104, but is a nonresponder. More extensive serological analysis and peptide mapping comparisons will be necessary to establish whether the *A* alleles of the B10.STA10 and B10.LIB55 strains are truly identical.

The concordance of *Ir* and *Ia* typing could mean either that the two types of loci are in strong linkage disequilibrium or that they are identical. The *H-2* haplotypes of the B10.W lines have been derived from wild mice most of which were captured in the area of Ann Arbor, Michigan. There is no known relationship between these wild mice and the ancestors of the present-day inbred strains (Zaleska-Rutczynska and Klein 1977). Since the two were most probably derived from two independent

populations, linkage disequilibrium due to common origin is extremely unlikely. Although some linkage disequilibrium between *H-2* loci does exist even among unrelated wild mice (Wakeland and Klein 1979), it does not appear to be so strong as to account for the observed concordance between the *Ia* and *Ir* typing results. Particularly informative in this respect are the strains B10.CHA2 and B10.STA62, which are responders to GA and share with the responder inbred strains only the *A* allele but differ from them at other *H-2* loci. Because of these relationships, the concordance in typing most likely reflects identity of *Ia* and *Ir* loci.

The second set of observations indicating identity of *Ia* and *Ir* loci concerns the blocking of the in vitro response with monoclonal antibodies against Ia antigens. That the *Ir*-controlled response can be blocked with anti-Ia sera was demonstrated previously, in particular by Shevach and co-workers (1972) working with guinea pigs, and Schwartz and co-workers (1976 a, 1978) working with mice. However, in these studies the source of the antibodies were conventional antisera and one could argue, therefore, that the inhibiting factors were not antibodies against *Ia*, but serologically unidentified antibodies against the hypothetical Ir-gene products. Since we used monoclonal antibodies, such an argument does not apply here. Our results demonstrate that monoclonal Ia antibodies inhibit the *Ir*-gene controlled T-cell responses in a subregion-specific manner. The response to GA controlled by the *A* subregion was blocked by antibodies against the A molecule, but not by antibodies against the E molecule (Fig. 1), and the opposite pattern of inhibition was obtained in the case of the anti-GLT[15] response (Fig. 2). The response to the latter antigen is under dual genetic control with one *Ir* locus residing in the *I-A* and the other in the *I-E* subregion. This dualism strikingly parallels the finding that the Ia-bearing E molecule is also controlled by two loci, one in the *I-A* and the other in the *I-E* subregion (Jones et al. 1978). Our observation that the anti-GLT[15] response can only be inhibited by an antibody against the E molecule demonstrates that the complex of E_α and E_β Ia-chains (controlled by the *E* and *A* subregions, respectively) is involved in the response. Blocking of one chain (probably E_α which carries the Ia.m7 determinant) apparently blocks sterically the recognition of the whole $E_\alpha E_\beta$ complex. Based on this finding, it is predictable that antibodies against determinants on the E_β chain would also inhibit T-cell responses controlled by two complementing *Ir* genes. In fact, it is probably the highly polymorphic E_β chain (Uhr et al. 1979), that is relevant to the *Ir* phenomenon, since differences in the immune response could not arise, if the product of the virtually monomorphic E_α locus were involved. The finding that several inbred strains (Table 3) and B10.W lines (data not shown) expressing the Ia.7-positive E_α chain (in combination with different E_β chains) are nonresponders to GLT[15] also argues against direct involvement of the E_α chain.

The results demonstrating inhibition of the anti-GLT[15] response only by anti-Ia.m7 (Fig. 2) are seemingly contradictory to the data of Schwartz and co-workers (1978), who showed blcoking of the dual *Ir*-gene controlled T-cell response to GLO with anti-Ia sera directed against either I-A- or I-E/C-subregion products. However, to detect I-A-subregion products, these authors used a $(B10 \times A)F_1$ anti-B10.D2 alloantiserum, which might also have contained antibodies against determinants on the E_β chain of the $H\text{-}2^d$ haplotype. This assumption is supported by their finding that the inhibitory capacity of this serum could not be removed by

absorption with B10.GD cells, which express A^d molecules but fail to express the E^d_β chain for lack of the appropriate E_α chain. Thus the antibodies believed to react with the A molecule were in fact directed against the E_β chain.

The results presented here do not establish the cellular level at which Ia antibodies exert their blocking effect on T-cell proliferative responses. However, from the fact that Ia-positive antigen-presenting cells are necessary for this type of response (Rosenthal and Shevach 1973, Waldron et al. 1973, Thomas et al. 1977, Yano et al. 1977), and that *Ir*-gene expression is also localized to these cells (Schwartz et al. 1979 b, Longo and Schwartz 1980), we conclude that antibody-blocking most likely occurs via Ia determinants on the antigen-presenting cell. Thus our data give strong support to the notion that Ia antigens as restriction elements for T-cell recognition are inseparable from the *Ir* phenomenon, at least in the case of T-cell responses controlled by genes in the *I-A* and *I-E* subregions.

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